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SYNERGISTIC EFFECTS OF EARTHWORMS AND SOIL MICROORGANISMS ON LITTER DECOMPOSITION IN SUDANO-GUINEA SAVANNAH ZONE OF NGAOUNDERE, CAMEROON

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The impact of interaction of functional groups of soil organisms on litter decomposition in the tropical savannahs is poorly investigated. Microcosm experiments on synergistic effects of earthworms and soil microorganisms on litter decomposition was conducted in laboratory in the constant conditions of temperature and humidity on leaf litters of six contrasting plant species of sudano-guinea savannahs of Ngaoundere, Cameroon. These are Anacardium occidentale L., Annona senegalensis Pes., Combretum molle R. Br. Ex G. Dom., Syzygium guineense var. guineense (Willd) D.C., Syzygium guineense var. macrocarpum (Engl.) F. White and Ximenia americana L. Samples of each plant species have been incubated in microcosms on four types of soil: 1) sieved soil without earthworms (NS), 2) sieved soil with earthworms (NE), 3) sieved and sterilized soil without earthworms (SS) and 4) sieved and sterilized soil with earthworms (SE). For each treatment, 60 samples of 7±0.01q of each litter types were weighed and placed on the top soil of microcosms. Three samples of each plant species at each treatment were removed at 15, 30, 50, 70 and 120 days. Their dry mass remaining and decay rate were determined. Results showed that during 120 days of incubation, dry mass remaining varied from 4.96% in X. americana for the treatment with sieved soil containing earthworms (NE) to 87.21% in S. guineense var. macrocarpum for treatment with sterilized soil without earthworms (SS). Litter decomposition rate was significantly and negatively correlated with polyphenol compounds at all treatments. Inversely no correlation was found with lignin. Relative synergistic effects of earthworms and microorganisms to litter decomposition ranged from 12.73% in S. guineense. var. guineense to 44.75% in X. americana according to plant species and were controlled by physico-chemical characteristic of litter. In short, interaction of earthworms and microorganisms provided a significant contribution to litter decomposition in the Ngaoundere savannahs. These results showed the importance of preserving all soil fauna diversity in this region for assuring efficiently nutrient cycling and contributing to soil fertility management.

Keywords: Earthworms, Litter decomposition, Synergistic effect, Microorganisms, Sudano-guinea savannah, Ngaoundere, Cameroon.

INTRODUCTION

decomposition process represents Litter an essential phase of the organic matter and nutrient cycle (Leon and Nelson, 2014). Litter decay rate is a factor that largely determines forest soil fertility and its regulation plays an important role in ecosystem functioning (Swift et al., 1979) as primary production and nutrient cycling (Cardinale et al., 2006; Elser et al., 2007). Most of the plant litter from above ground plant production is supplied in the form of leaves, which decompose much faster than the woody litter components produced both above- and belowground in ecosystems. Numerous factors, including resource quality (Maisto et al., 2011; Parsons et al., 2014), climate (Kurzatkowski et al., 2004; Dhanya et al., 2013; Suseela et al., 2013; Bothwell et al., 2014) and soil nutrient availability (McClaugherty et al., 1985; Li et al., 2011) for plant growth and forest disturbance (van Dam, 2001; Ibrahima et al., 2002; Ibrahima et al., 2010) have been recognized to strongly influence plant litter decomposition rates. The relative importance of these factors varies according to the characteristics of the ecosystems under consideration (Lavelle et al., 1993; Trofymow et al., 2002).

Other significant variables that affect litter decomposition process are soil organisms, which are diverse and abundant (Carcamo et al., 2000; David and Gillon, 2002). Representatives of nearly all groups of terrestrial invertebrates are involved in litter decomposition and the subsequent release of nutrients (Carcamo et al., 2000; David and Gillon, 2002). Soil microorganisms (<0.2 mm; including bacteria, fungi, protozoa) are directly responsible for most litter decomposition, and soil macroorganisms (>2 mm; including termites, earthworms and macroarthropods) can stimulate decomposition via litter fragmentation and defecating into the soil, and through altering the activity and composition of the microbial communities (Wardle, 2002; Rawlins et al., 2006; Meyer et al., 2013). Soil faunal excretions like mucus and other substances provide particularly suitable substrates for microbial communities (Kautz and Topp, 2000; Lavelle and Spain, 2002; Wardle, 2002). By the processes of fragmentation and comminution, earthworms can influence litter decomposition through their pedological effects:

through modification of soil structure construction of burrows enhancing and the decomposition of plant debris through the burial of leaves (Lavelle, 1988; Swift and Anderson, 1989). Madge (1965) showed that the earthworms affect litter decomposition not by pulling leaves into its galleries but by covering them with casts. Marinissen and Bok (1988) demonstrated that the activity of earthworms could lead to increases in the population size and abundance of Collembola. Brown (1995) asserted that the effect of earthworms on microorganisms could be a net reduction in their and density species diversity due competitiveness of the interaction.

The role of microorganisms in litter decomposition cannot be discussed without reference to their interactions with the soil fauna. Interactions between soil organism communities as earthworms and soil microorganisms in the litter decomposition process have been well studied for tropical forest and temperate ecosystems (Heemsbergen et al., 2004; Hättenschwiler and Gasser, 2005; Bastian et al., 2008; McKie et al., 2008; De Oliveira et al., 2010). However, little information was available for tropical savannahs and the complex interactions between soil fauna and microorganisms are less well understood (Adejuyigbe et al., 2006). The objective of the study was to determine the effects of earthworms and its relative synergistic effects with microorganisms on leaf-litter decomposition of six contrasting plant species of the sudano-guinea savannahs zone of Ngaoundere Cameroun.

MATERIALS AND METHODS

Study Area

The locality of this study lying within the Adamawa region located on 7°25'N and 13°33'E, with elevation of about 1081 m. The climate is humid sudano-guinea with two seasons: a long dry season (from November to March), rainy season (from April to October). Mean annual rainfall is about 1500 mm and mean annual temperature 23°C (Humbel, 1971). The soil in majority is red ferralitic developed on old basalt. The dominant vegetation is woody and shrubby Savannahs (Letouzey, 1968).

Leaf Selection

In this study, only fresh fallen leaf litter of six socioeconomical plant species of the Sudano-guinea

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Ngaoundere of used. The savannahs was experiments involved six species: deciduous broadleaved including four shrub species (Annona senegalensis. molle. Syzygium Combretum guineense Ximenia var. macrocarpum and americana) evergreen and an broad-leaved including one tree species (Syzygium guineense var. guineense) and a shrub species (Anacardium occidentale). The distribution area of S. guineense var. quineense (Willd) D.C. (Myrtaceae) is the forest gallery, while A. occidentale L. (Anacardiaceae), A. senegalensis Pes. (Annonaceae), C. molle R. Br. Ex G. Dom. (Combretaceae), S. guineense var. macrocarpum (Engl.) F. White (Myrtaceae) and X. americana L. (Olacaceae) are located in the upland Savannahs. They are source of income, food, firewood, medicinal substances and soil fertility indicators for the farmers of this region (Yonkeu, 1993; Ibrahima et al., 2007). The farmers start now to conserve these plant species in their farms and gardens. New litter fall samples were collected directly from forest floor in the Ngaoundere humid savannahs, next to the Dang campus of the University of Ngaoundere, Cameroon, during maximum leaf fall period (November 2006 – January 2007) that corresponds to dry season and soil was very dry. No leaching occurred from new litter which was sorted, air-dried and stored in the laboratory before use.

Leaf Litter Incubation Experiment

Study was carried out in microcosms at the constant laboratory conditions. Microcosm model used is of the type described by Taylor and Parkinson (1988) and as modified by Gillon et al., (1994). One kg of soil consisting of surface organic horizon from the Dang site was placed in each of microcosm. Characteristics of site including soil type were described earlier by Ibrahima et al., (2010). In order to get rid of lumps and vegetation remains, the soil was previously sieved at 1 mm to eliminate all meso- and macro-fauna then a part of it was sterilized during 24h by the dry heat method (Adejuyighe et al., 2006).

In an attempt to determine the relative effects of the two types of soil organisms (earthworms and microorganisms) on litter decomposition rate, four different treatments were realized in similar incubation chambers: 1) microcosms containing only

sieved and sterilized soil without earthworms (SS); 2) microcosms containing sieved and non-sterilized soil without earthworms (NS); 3) microcosms containing sieved and sterilized soil with common earthworms (SE) of the region and 4) microcosms containing sieved and non-sterilized soil with earthworms (NE). We assumed the sieved and nonsterilized soil containing soil microorganisms. The litter mass loss of microcosms containing only sieved and sterilized soil without earthworms (SS) was due to leaching.

For each treatment, ninety (90) samples of 7.00±0.01g of each of six litter types (A. occidentale, A. senegalensis, C. molle, S. guineense var. quineense, S. quineense var. macrocarpum and X. americana) were weighed and placed on the soil top of microcosms and material of 2 mm mesh to recover all the material at each sampling time. The quantity of water needed to replace that evaporating and thus maintain constant soil moisture during incubation was also calculated each week by weighing the microcosms and adding. microcosms were maintained at 23°C ± 1°C and a relative humidity of 68% ± 2% throughout the experiment.

Three samples of each species at each treatment were taken at 15, 30, 50, 70 and 120 days, and their mass determined by electrical balance (Sartorius Type ISO-9001), after drying in oven, Memmert, for 48 hours at 59°C. Three additional litter samples were weighed after drying in an oven at 59°C for 48 hours to determine the original litter dry mass. The litter dry mass remaining (LMR) as a percentage of the initial mass was calculated as following: LMR (%) = (DM_t/DM_0) x100 where, DM_t is dry mass at the sampling time t and DM₀, the initial dry mass.

The contribution of earthworms in presence (EM) and absence of microorganisms (EO) and that of soil microorganisms in presence (ME) and absence of earthworms (MO) were calculated as following:

(Effects of earthworms in EM = NE - NS; presence of soil microorganisms); (Effects of earthworms in EO = SE-SS: absence of soil microorganisms) ME = NE-SE: (Effects of soil microorganisms in presence of earthworms) (Effects MO = NS-SS; of soil microorganisms in absence of earthworms)

SY = NE-SS (Synergistic effects of earthworms and soil microorganisms)

For each species, three replicates of litter samples were oven-dried (48 h at 65°C), then weighed and their thickness measured by calliper. To avoid fragmentation, leaf litter was moistened again, spread out and then the leaf side length and maximum width were measured using millimeter paper. Litter area and surface mass (Gillon et al., 1994) or sclerophylly index (Choong et al., 1992) were calculated using respectively equation of Paynes et al., (1997) and, litter mass and area ratio

A = 0.68 x (length x maximum width) - 0,114SI = DM/A

Where, A, SI and DM are respectively leaf litter area (cm²), sclerophylly index (mg.mm⁻²) and dry mass of initial litter (g).

Chemical Analysis

The samples of initial dry litters were ground in *Micro Hamer Mill Culatti Blender* and sieved at 1 mm. The concentration of cellulose, phenolic compounds and lignin were respectively determined by colorimetric method (Updegraff, 1969), by Folin-Ciocalteu reagent (Marigo, 1973) and by van Soest's (1965) and detergent method.

Data Analysis

The litter dry mass remaining (LMR) of each species for each treatment in relation to litter incubation time (in days) was fitted to the following simple exponential model:

LMR = A*exp(-kt)

Where, k is the decomposition rate constant (%.day⁻¹), A the compartment of water soluble substances and other compounds. Because of the high significance of coefficients of determination, the equation was adoptee.

A multiple comparison among the decomposition rate constants (k) was carried out using the T'-method (Sokal and Rohlf, 1981) to compare for each species the effects of soil organisms (earthworms and microorganisms) on litter decomposition rate.

Using one-way ANOVA (species or soil organisms), following by *Scheffe's* mean comparison test at 5% (if ANOVA was significant)

compared LMR among plant species and among treatments (SS, SN, SE and NE). ANOVA was also used to test the effects of treatments on LMR at each sampling time (15, 30, 50, 70 and 120 days). Two-way ANOVA (species and treatments) was used to compare the combined effects of litter types and treatments. Pearson's correlation coefficients decomposition rate were calculated between constants (k) and physico-chemical properties of initial litter. Simple and multiple (stepwise) regression models were also used to determine relationships between these parameters. These tests were conducted through software package SX for DOS, version 4.0. (Statistics, 1992).

RESULTS

Initial Litter Properties

All initial litter characteristics presented in this study differed significantly among plant species (Table 1). The highest values of litter thickness (0.78 mm) and area (58.98 cm²) were found in A. senegalensis shrub and the lowest ones in X. americana shrub (0.34 mm and 22.78 cm²). Sclerophyllous index (SI) varies significantly from A. occidentale (0.15 mg.mm⁻²) to A. senegalensis (0.23 mg.mm⁻²) and X. americana (0.25 mg.mm⁻²). The organic compounds as cellulose (2.89%) and phenolic compounds (6.90%) were highest in X. americana and lowest molle (1.14%) respectively in C. senegalensis (1.16%). The highest values of lignin were in A. senegalensis (6.03%) and the lowest one in S. guineense var. guineense (2.84%).

Changes in LMR during course of litter incubation time

The dynamics of LMR during 120 days of microcosms at constant conditions of laboratory were similar for all species at each treatment, except those of *X. americana* (Figure 1). The differences of LMR between *X. americana* and others species were started since the first sampling time (15 days) for both treatments with no sterilized soil and with and without earthworms (NE and NS).

The Changes of LMR differed significantly among treatments (Figure 2). From the second to the end sampling time, two types of patterns of LMR

6.90 (0.23)a

1014.07***

Species	Thickness (mm)	Area (cm²)	IS (mgmm ⁻²)	Cellulose (%)	Lignin (%)	Phenol (%)
AO	0.43 (0.07)c	51.83 (14.20)ab	0.15 (0.05)b	ND	ND	ND
AS	0.78 (0.10)a	58.98 (19.46)a	0.23 (0.08)a	2.02 (0.06)ab	6.03 (0.69)a	1.16 (0.02)e
CM	0.62 (0.09)b	42.97 (11.35)b	0.18 (0.06)ab	1.14 (0.41)b	5.34 (0.78)ab	2.62 (0.09)d
SG	0.56 (0.10)b	29.10 (8.36)c	0.20 (0.06)ab	1.57 (0.25)ab	2.84 (0.68)b	4.79 (0.35)b
SM	0.39 (0.06)c	49.75 (14.70)ab	0.21 (0.08)ab	1.80 (0.19)ab	5.77 (0.74)ab	3.66 (0.01)c

0.25 (0.06)a

6.06**

2.89 (0.48)a

8.34*

4.65 (0.12)ab

7.75*

0.34 (0.03)c

90.36***

XA

F

22.78 (5.21)c

22.57***

Table 1. Physical and chemical properties of initial litters of six plant species of Ngaoundere savannahs of Cameroon.

p<0.05, ** p<0,01, ***p<0,001. Numbers in parentheses indicate standard deviation. Non determined value (ND). Different letters indicate significant differences among different species : A. occidentale (AO), A. senegalensis (AS), C. molle (CM), S. guineense var. guineense (SG), S. guineense var. macrocarpum (SM) and X. americana (XA).

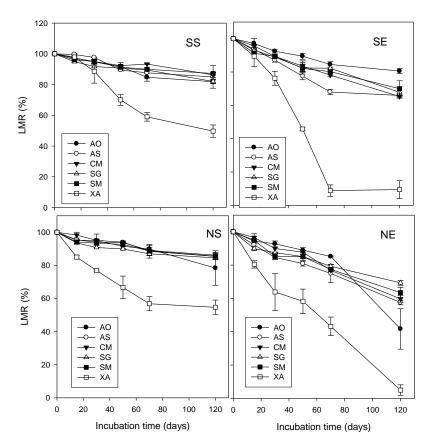


Figure 1. Changes of LMR of six species at each of four treatments (SS, NS,NE and SE) during the course of incubation time in laboratory microcosms. Non sterilized soil without earthworms (NS), sterilized soil without earthworms (SS), non sterilized soil with earthworms (NE), sterilized soil with earthworms (SE). A. occidentale (AO), A. senegalensis (AS), C. molle (CM), S. guineense. var. guineense (SG), S. guineense. var. macrocarpum (SM) and X. americana (XA).

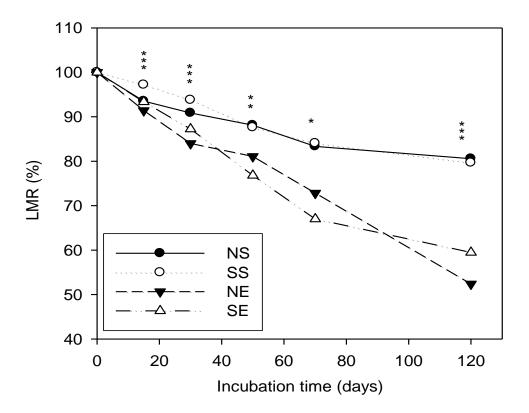


Figure 2. Changes of LMR mean of six species of treatments with (NE and SE) and without (SS and NS) earthworms during the course of incubation time in laboratory microcosms. * p<0.05, ** P<0.01 and *** P<0.001. Non-sterilized soil without earthworms (NS), sterilized soil without earthworms (SS), non-sterilized soil with earthworms (NE), sterilized soil with earthworms (SE).

changes were found. The LMR for the treatments with earthworms (SE and NE) were significantly low than those of treatments without earthworms (SS and NS) during course of incubation time. In each group, the patterns of LMR changes were similar. The patterns of LMR of the treatments for each species were presented in Figure 3. According to treatments, three groups of species were found. The group constituted by A. occidental showed no significant difference of LMR changes among four treatments, except at the last sampling time (120 days) where the LMR for the only treatment with no sterilized soil containing earthworms (NE) was significantly low than the three others treatments. For the second group including all the remaining species, LMR for the treatments with earthworms (SE and NE) were significantly low than those of treatments without earthworms (SS and NS) during course of incubation time.

The LMR was fitted to negative exponential model, with highly significant coefficient of correlation at all species and all treatments (Table 2). A multiple comparison of decomposition rate constants (k) by T'-method (Sokal and Rohlf, 1981) showed that the decay rates varied among species and among treatments (Figure 4). X. americana had higher decomposition rate constant than the five others species for all the treatments. The decomposition rate constants of all species for both the treatments without earthworms (SS and NS) were similar and lower than those of both the treatments with earthworms which were not differed between them. That of A. occidentale was significantly higher for the treatment with no sterilized soil containing earthworms (NE) than those of other litter. For all species, the decomposition rate constants for treatments with earthworms were significantly higher than for treatment without earthworms, excepting

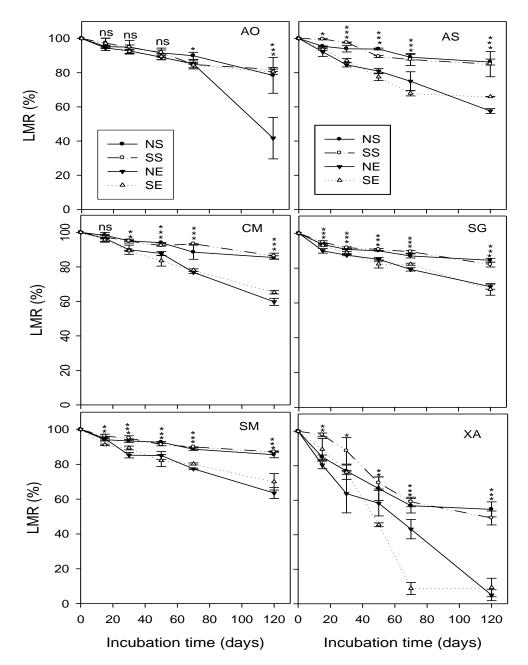


Figure 3: Changes of LMR (%) of each of six species for four treatments during the course of incubation time in laboratory microcosms. ns, non-significant, * p<0.05, ** P<0.01 and *** P<0.001. Non-sterilized soil without earthworms (NS), sterilized soil without earthworms (SS), non sterilized soil with earthworms (NE), sterilized soil with earthworms (SE). *A. occidentale* (AO), *A. senegalensis* (AS), *C. molle* (CM), *S. guineense.* var. *guineense* (SG), *S. guineense.* var. *macrocarpum* (SM) and *X. americana* (XA).

that of A. occidentale for treatment sterilized soil containing earthworms (SE) which was statistically

similar with those for both treatments without earthworms (SS and NS).

Table 2. Coefficient of correlations (R) of exponential regression equations ($y = \exp(-kt)$) describing changes in LMR with incubation time (in days). All are highly significant at P< 0.001, n = 18 or * n = 17.

Species	SS	NS	SE	NE
A. occidentale	0.9476	0.8511	0.9587	0.8713
A. senegalensis	0.8785*	0.9368*	0.9580*	0.9806
C. molle	0.9549	0.9385	0.9944	0.9794
S. guineense . var. guineense	0.9642	0.9126	0.9775	0.9758
S. guineense. var. macrocarpum	0.9476*	0.9368	0.9629	0.9803
X. americana	0.9592*	0.9382*	0.9467	0.9570

Sterilized soil (SS), non sterilized soil (NS), sterilized soil with Earthworms (SE) and non sterilized soil with Earthworms (NE)

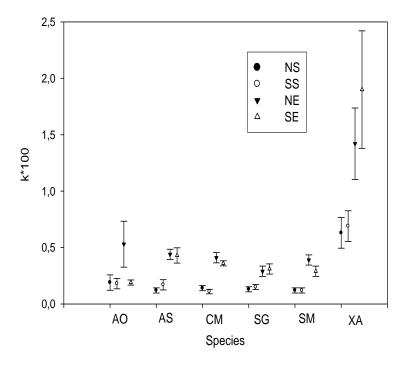


Figure 4. Decomposition rates constant (k) of 6 species for each treatment after 120 days of litter incubation laboratory microcosms. Treatments: non sterilized soil (NS) and sterilized soil (SS) without earthworms; non sterilized soil with earthworms (NE) and sterilized soil with earthworms (SE). A. occidentale (AO), A. senegalensis (AS), C. molle (CM), S. guineense. var. guineense (SG), S. guineense. var. macrocarpum (SM) and X. americana (XA).

LMR at the last sampling date

At each of four treatments, litter types (or species)

differed significantly by their LMR at the end of incubation (120 days) in microcosms (Table 3). The LMR varied from 49.71% in *X. americana* to 87.21%

Species	SS	NS	SE	NE	F (Treatments)
AO	81.89 (1.12)a α	78.40 (10.47)a α	80.56 (1.5)a $lpha$	41.69 (12.12)c β	17.27***
AS	84.98 (7.35)a α	86.20 (1.72)a α	65.95 (0.11)b β	57.57 (1.38)b β	50.90***
CM	86.33 (1.66)a α	85.41 (1.01)a α	65.28 (0.96)b β	59.75 (2.03)ab δ	254.22***
SG	82.07 (1.40)a α	84.55 (1.07)a α	67.81 (3.34)b β	69.34 (1.47)a β	53.83***
SM	87.21 (0.52)a α	85.58 (1.79)a α	69.98 (4.73)b β	63.50 (3.13)abβ	36.27***
XA	49.71 (4.03)b α	54.69 (4.37) b α	9.39 (5.30)c β	4.96 (6.17)d β	58.27***
F (Species)	46.51**	14.16***	161.25***	49.72***	

Table 3. Litter mass remaining (LMR in %) at the end of incubation time (120 days) of the six species (AO, AS, CM, SG, SM and XA) at each of the four treatments (SS, NS, SE and NE).

in S. guineense var. macrocarpum for the treatment with sterilized soil and without earthworms (SS). from 54.69% in X. americana to 86.20% in A. senegalensis for treatment with no sterilized soil and without earthworms (NS), from 9.39% in X. americana to 80.56% in A. occidentale for the treatment with sterilized soil and earthworms (SE) and from 4.96 in X. americana to 69.34% in S. quineense var. quineense for the treatment with no sterilized soil and earthworms (NE). Globally, litter mass loss was the significantly highest in X. americana for both treatments with earthworms (SE and NE), that of litter of A. occidentale for treatment with sterilized soil and earthworms (SE) and that of S. guineense var. guineense litter for treatment with no sterilized soil and earthworms (NE) were the contrary, lowest. for treatment without earthworms (SS and NS), litter mass loss of X. americana was higher than those of the five others species which did not differ significantly among them. Two-ways ANOVA showed that combined effects of species and treatments was high significant (F= 10.93 and P<0.001) on LMR at the end of incubation time (120 days).

Contribution of soil organisms

Relative contributions of earthworms, microorganisms and litter leaching to litter mass loss

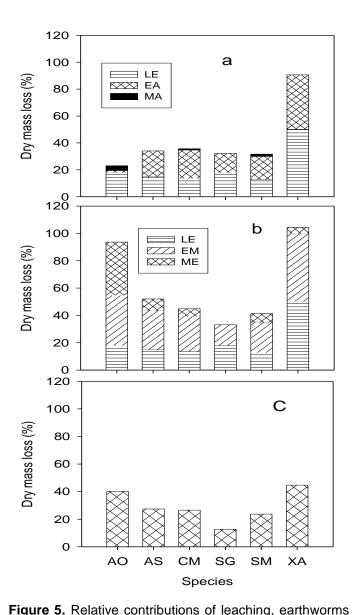
were illustrated by Figures 5a; b and c. Litter mass loss for treatment with sterilized soil and without earthworms (SS) would correspond to litter leaching. The percentage of leaching of the six species varied from 12.79% in S. guineense var. macrocarpum to 50.29% in X. americana (Figure 5a). This leaching was significantly higher in X. americana than the other five species which happened to be similar among them.

The contribution of earthworms without and with microorganisms to litter decomposition varied according to species (Figure 5a and b). This relative contribution without microorganisms (EA) ranged from 1.34% in A. occidentale to 40.23% of litter mass loss in X. americana (Figure 5a). On the contrary, with the presence of microorganisms (EM), the relative contribution of earthworms to litter decomposition increased for all species, particularly for A. occidentale where it passed from 1.34% for earthworms alone (EA) to 36.71% for earthworms with organisms (EM).

The effects of microorganisms without earthworms (MA) appeared only in *C. molle*, *S. guineense* var. macrocarpum and A. occidentale, with low rate values of 0.92%, 1.63% and 3.50% respectively (Figure 5a). On the contrary, with presence of earthworms, the relative effects of microorganisms on litter decomposition appeared for all species with sometime the high values, except for S. guineense

^{**} P<0.001, ***P<0.001. Numbers in parentheses indicate standard deviation. Different arabic letters in columns indicate significant differences among different species. Different Greek letters in rows indicate significant differences among treatments (SS, NS, SE and NE). A. occidentale (AO), A. senegalensis (AS), C. molle (CM), S. guineense var. guineense (SG), S. guineense var. macrocarpum (SM) and X. americana (XA). Sterilized soil (SS), non sterilized soil (NS), sterilized soil with Earthworms (SE) and non sterilized soil with Earthworms (NE).

(XA).



and microorganisms to litter decomposition after 120 days of litter incubation laboratory microcosms.

a: Litter leaching (LE), effects of Earthworms alone (EA) and microorganisms alone (MA). b: Effects of earthworms in presence of microorganisms (EM) and effects of microorganisms in presence of Earthworms (ME); c: synergic effects. A. occidentale (AO), A. senegalensis (AS), C. molle (CM), S. guineense. var. guineense (SG), S. guineense. var. macrocarpum (SM) and X. americana

var. *guineense* (Figure 5b). The contribution of microorganisms to litter decomposition of *A. occidentale* for example was 10 times higher with

the presence of earthworms (38.87%) than without earthworms (3.50%). The relative contribution of microorganisms with earthworms ranged from 4.43% in *X. americana* to 38.87% in *A. occidentale*. Synergistic effects of earthworms and microorganisms to litter decomposition differed among species (Figure 5c), with the values ranged from 12.73% in *S. guineense* var. *guineense* to 44.75% in *X. americana*. The synergistic effects of microorganisms and earthworms were not the sum of individual effects of both the soil organism groups.

Correlation between LMR, decomposition rate constant and initial litter properties

Relationships between LMR at the end of incubation time and initial litter properties varied according to treatment (Table 4). For each of the four treatments, LMR after 120 days of incubation was correlated significantly and negatively with phenolic compounds. On the contrary, no significant correlation was found between LMR and lignin for each of four treatments. For sclerophyllous index (SI), LMR was correlated negatively with it only at treatment with sterilized soil containing earthworms (SE). It was found that LMR was correlated positively with thickness only at the treatments with microorganisms (NS and NE).

Pearson coefficient correlations showed relationships between litter decomposition rate constants (k) and the mean values of initial litter properties (Table 5). These relationships varied according to treatments. Phenolic compounds were correlated significantly and negatively with litter decomposition rate constants (k) only at the treatments with earthworms (SE and NE). For the treatment with sterilized soil and without earthworms (SS), there were no significant correlations between litter decomposition rate constants (k) and the initial litter properties. On the contrary, for the others treatments (NS, SE and NE), the significant correlation were observed. For the treatment with no sterilized soil and earthworms (NE), k was correlated significantly with three initial litter properties, one physical (litter area) and two chemical (lignin and phenolic compounds). For each of the remaining treatments (NS and SE), k was correlated significantly with two initial properties, one physical and one chemical, that is

Table 4. Simple linear regression equations between LMR at the end of incubation time (120 days) and initial litter properties (Thickness, area, sclerophyllous index, cellulose, lignin and phenolic compounds) at each of the four treatments (SS, NS, SE and NE).

Treatments	Linear regressions equations	R ²	N	F
	LMR = 57.769+42.088*Thickness	0.2441	17	4.20ns
	LMR = 53.319 + 0.6166*Area	0.3936	17	8.44*
SS	LMR = 100.18 + 106.92*SI	0.0704	17	0.98ns
	LMR = 112.882 - 19.268*Cellulose	0.7021	8	14.14**
	LMR = 107.374 – 7.288*Phenolic Compounds	0.7808	8	21.37**
	LMR = 70.363 + 1.446*Lignin	0.010	8	0.06ns
	LMR = 62.166 +34.806*Thickness	0.2523	17	5.06*
	LMR = 62.371+0.4185*Area	0.2703	17	5.56*
NS	LMR = 100.38-98.160*SI	0.1373	17	2.39ns
	LMR = 103.43 - 12.240*Cellulose	0.4109	9	4.88ns
	LMR = 94,220 – 3.473*Phenolic Compounds	0.4676	9	6.15*
	LMR = 78.693 + 0.7082*Lignin	0.111	9	0.08ns
	LMR = 27.887+62.1594*Thickness	0.1478	17	2.60ns
	LMR = 9.2950+1.1908*Area	0.4646	17	13.02**
SE	LMR = 141.55-410.12*SI	0.4440	17	11.98**
	LMR = 109.505 - 27.918*Cellulose	0.6015	9	12.08**
	LMR = 89.482 – 8.523*Phenolic compounds	0.5503	10	9.79*
	LMR = 45.820 + 2.252*Lignin	0.0152	10	0.12ns
	LMR = 11.329+73.541*Thickness	0.2619	18	5.68*
	LMR = 19.352 - 0.7085*Area	0.1836	18	3.60ns
NE	LMR = 81.068 + 156.17*SI	0.0750	18	1.30ns
	LMR = 111.421 – 31.751*Cellulose	0.7144	9	20.03**
	LMR = 81.922 – 7.933* Phenolic Compounds	0.4377	10	6.23*
	LMR = 56.262 – 0.946*Lignin	0.0025	10	0.02ns

ns: non-significant, * P<0.05; ** P<0.01; ***P<0.001. Sterilized soil (SS), non sterilized soil (NS), sterilized soil with Earthworms (SE) and non sterilized soil with Earthworms (NE).

sclerophyllous index and cellulose for treatment with no sterilized soil and without earthworms (NS) and, thickness and phenolic compounds for treatment with sterilized soil and earthworms (SE).

The stepwise model showed the relationships between LMR at the end of litter incubation and associations of initial litter parameters at each of the four treatments (Table 6). The relationships between LMR and initial litter properties was explained more than 86% by the association of three chemical parameters (lignin, cellulose and phenolic compounds) and one physical parameter (sclerophyllous index) for both the treatments with soil microorganisms (NS and NE) or two physical parameters (sclerophyllous index and thickness) for the treatments without soil microorganisms and with earthworms (SE). In reverse, for the treatments without earthworms and soil microorganisms (SS), this relationship was explained about 95% by the association of one chemical parameter (lignin) and three physical parameters (sclerophyllous index, thickness and area).

DISCUSSION

Many studies reported on the synergistic effects of earthworms and other soil organisms including

Table 5. Correlation coefficient of Pearson (n=5) calculated between litter decomposition rate constants (k) and initial litter properties (Thickness, area, sclerophyllous index, cellulose, lignin and phenolic compounds) at each of the four treatments (SS, NS, SE and NE). In bold significant (P< 0.05 or P<0.01) correlations.

Parameters	SS	SV	NS	NV
Thickness (mm)	0,6367	0,9510	0,0810	0,4146
Area (cm ⁻²)	0,2197	0,6163	-0,5690	0,9190
Sclerophyllous index (g mm ⁻²)	0,7850	0,4235	-0,9199	0,3079
Cellulose (%)	0,7215	0,2631	-0,9693	0,2150
Lignin (%)	-0,1351	0,4595	-0,3483	0,9599
Phenolic compounds (%)	-0,3019	-0,9051	0,1933	-0,9193

Sterilized soil (SS), non-sterilized soil (NS), sterilized soil with Earthworms (SE) and non sterilized soil with Earthworms (NE).

Table 6. Multiple regressions (Stepwise) between LMR at the end of incubation time (120 days) and initial litter properties (Thickness, area, sclerophyllous index, cellulose, lignin and phenolic compounds) at each of the four treatments (SS, NS, SE and NE).

Treatments	Stepwise regression equations	R ²	n
NS	LMR = 74.519 – 4.501*lignin + 3.969*Cellulose – 3.301* Phenol + 27.221*SI	0.9159	9
SS	LMR = 122.787 - 6.467*Lignin - 354.496*SI + 48.723*Thickness + 0,702*Area	0.9533	8
NV	LMR = 65.180 - 12.658*Lignin + 8.678*Cellulose - 7.280*Phenol + 100.200*SI	0.8658	10
SV	LMR = 85.725 – 11.202*Lignin + 7.897*Cellulose – 11.034*Phenol + 113.237*SI– 44.946*Thickness	0.8828	10

Sterilized soil (SS), non sterilized soil (NS), sterilized soil with Earthworms (SE) and non sterilized soil with Earthworms (NE).

microbes and fungi on litter decomposition process in terrestrial and aquatic ecosystems (Lussenhop, 1992; Suberkropp, 1992; Jonsson and Malmqvist, 2000; Wall et al., 2001; Heemsbergen et al., 2004; Hättenschwiler and Gasser, 2005; Hättenschwiler et al,. 2005; Tiunov and Scheu, 2005; Zimmer et al,. 2005; Duffy et al., 2007; Cardinale et al., 2009; Reiss et al., 2009: Rotherav et al., 2009: Leon and Nelson, 2014; Parsons et al., 2014; Suseela et al., 2013; Dhanya et al., 2013; Bothwell et al., 2014). But the synergistic contribution of microorganisms and earthworms in leaf litter decomposition process in African Savannah has been rarely studied with, our knowledge, only an exception of Adejuyigbe et al., (2006) who asserted positive effects of the interaction of microarthropods and earthworms on the litter decomposition of Senna siamea in Savannah of Nigeria. The value reported in their study was about 61% of initial mass loss. After 120

days of six contrasting and co-occurring litter incubation in microcosms in our study, our findings have shown that the mass loss during litter decomposition in microcosms was higher with both organisms presence than with either soil earthworms nor soil microorganisms alone present. The value of this synergistic contribution varied from 12.73 to 44.75% of mass loss according to litter type. The difference between the results of two studies was due to difference of the methodology and litter quality. In fact, the microcosms in their study were consisting of Oxic Paleustaff soil (red earth) (USDA) and contained six individual of Earthworms (Hyperiodrilus sp.) microarthropods. Conversely, in our study, the microcosm soil was constituted of ferralitic soil (Humbel, 1971) and containing microorganisms and 4 individual of earthworms (Lombricus sp.). The litters used under both studies differed also by their

quality. Senna siamea litter was higher in lignin (9%) and lower of polyphenols (1.78%) concentrations than those of litters used in our study (lignin concentrations varied from 2.84 to 6.03% and polyphenols compounds from 1.16 to 6.90%). Others synergistic interactions among different species of litter feeding animals have also been observed for three Plecoptera (insects) species in an aquatic ecosystem (Jonsson and Malmqvist, 2000), for eight different soil macrofauna species (Heemsbergen et al., 2004), for the combination of an earthworm and an isopod species (Zimmer et al., 2005), and for complementarily of snail and millipede in the Mediterranean forest (De Oliveira et al., 2010). All these studies were done in controlled laboratory conditions like our study. The rare published field study manipulating macrofauna presence has shown no synergistic effects between Glomeris spp. and anecic earthworm species exposed to a range of different litter species and mixtures (Hättenschwiler and Gasser, 2005).

Soil fauna largely control the decomposition process through breakdown of litter, digestion, and stimulation of micro-organism activities (Byzov et al., 1996; Maraun and Scheu, 1996). In line with above findings, our study demonstrated that relative contributions of soil microorganisms (<1mm) and earthworms to litter decomposition process were significant and varied according to plant species. Indeed, the decompositions of 6 litters in the 2 earthworm-free treatments (SS and NS) do not differ significantly amongst them for all species. This suggests that the action of microorganisms alone on the litter decomposition, that is, their contribution in the absence of earthworms is very low for all species (Figure 5a). On the other hand, in the presence of earthworms, this contribution of microorganisms to the litter decomposition is wholly significant for the 6 litters. This confirms that the action of microorganisms would be tributary of that of the soil macrofauna just like termites and earthworms. Similarly, concerning treatments with earthworms (SV et NV), the mass loss of litters is significantly higher on the normal soil with earthworms than on the sterilized soil with earthworms, except for litter of S. guineense var. quineense and X. americana. For these two litters, the presence of microorganisms of the soil does not significantly increase the action of earthworms. These results suggest on the one hand that while the action of earthworms is important at the beginning of the litter decomposition, it must be completed by that of microorganisms including microbes and mushrooms in order to ensure a mineralization of nutrients and, on the other hand, these synergistic actions depend on the type of litter. It is clear that earthworms and microorganisms play synergistic roles in litter decomposition process and this could be attributed to differences in their functional dissimilarity (Heemsbergen et al., 2004; Gessner et al., 2010). In fact, the macrofauna just like earthworms start by fragmenting the litters, then expand their surface thereby allowing an easier access of microorganisms and microbes into the cells of leaves for the degradation of organic molecules (Swift et al., 1979; Adejuyigbe et al., 2006; De Oliveira et al., 2010). Other studies have also shown that earthworms can promote microbial abundance, diversity and activities by facilitating mechanisms that are:1) fracturing litter, thereby increasing the surface area available to microbes, 2) available nutrients through waste increasing production and mucus secretion, 3) consuming microbes, which can lead to vigorous microbial growth, 4) modifying the habitat in ways that facilitate microbial growth/activity and 5) modifying the habitat in ways that increase recruitment of other smaller invertebrates (i.e. <2mm) that can influence decomposition bγ the four mechanisms (Belovsky and Slade, 2000; Cardinale et al., 2007; Eisenhauer et al., 2007; De Oliveira et al., 2010). Unlike for microorganisms, it seems that soil macrofauna just like termites earthworms would not possess the necessary enzymes to directly degrade the organic compounds of the soil like the lignin, the tannin and the humic complex of the soil. Such enzymes would be produced by microorganisms and mushrooms and, these macrodetritivores to assimilate compounds and nutrients resulting from the fragments of ingested litters (Cardinale et al., 2007; De Oliveira et al., 2010; Crowther et al., 2012). Other possible explanation given by Gessner et al., (2010) is that colonization of leaf litter by palatable fungal species can render even relatively refractory leaf species attractive to macrodetritivores and stimulate consumption, as indicated by experiments in both terrestrial and aquatic ecosystems (Mulder et al., 1999; Lecerf et al., 2005; Hättenschwiler and Gasser, 2005; Bastian et al., 2008). These

mechanisms, so called facilitation and resource partitioning, are widely discussed in the literature of plant diversity effects on net primary production and are thought to contribute to complementary in more diverse plant communities (Cardinale et al., 2007). It is notoriously difficult to separate these mechanisms (van Ruijven and Berendse, 2003; Roscher et al., 2008) and it is likely all contributed to the observed increased litter mass loss in our study.

The synergistic effect of earthworms and other soil organisms such as microorganisms, microbes and mushrooms on the litter decomposition process varies according to the type of litters (Figure 3c). This effect is stronger on litters with low thickness and rich in cellulose (Table 1) and with high capacity to release water soluble substance (Ibrahima et al., 2003) as X. americana (44,75%) and A. occidentale (40,20%). Moreover, the synergistic value is lower than the sum of individual values of earthworms and soil microorganisms for all species. These results suggested that the physical features of leaf-litter as litter thickness, as well as their chemical quality as lignin and polyphenols seemed generally to play a great role in the faunal effects on litter decomposition process as reported by De Oliveira et al., (2010), Cleveland et al., (2002, 2006) and Kaspari and Yanoviak (2008) in tropical Forests. From their field study on sixteen co-occurring tropical rain forest tree species of French Guiana, Cog et al., (2010) suggested that animals feeding preferences among litter specie were apparently essential driven by litter condensed tannins Mechanisms similar concentration. to those proposed for the anti-herbivore effects of tannins may explain the avoidance of tannin-rich litter by soil animals. These potentially include toxicity or deterring effects (Poinsot-Balaguer et al., 1993) and tannin binding with dietary proteins or digestive enzymes (Feeny, 1969). In contrast to the negative correlation with litter condensed tannin, the fauna effect increased with increasing litter hemicelluloses and cellulose concentrations (Cog et al., 2010). We found significant (P<0.05) correlations between the remaining mass loss and polyphenol with and without earthworms presence of and microorganisms, and this remaining mass loss correlated also with litter thickness in treatment with microorganisms presence. However, as concerns the other physico-chemical characteristics of litters like the cellulose and the lignin, this correlation

varies according to the type of treatment. But it is noteworthy that the phenolic compounds, the lignin and the cellulose could not be determined in all litters, and this could shadowed the quality effect of the litter on the synergistic action of functional groups of soil organisms on the litter decomposition as per our research.

CONCLUSION

In conclusion, as far as we know, this study indicated that interaction of earthworms and microorganisms provided a significant contribution to decomposition of litters of different co-occurrence tree species of Ngaoundere savannahs used in this These explained by complementarily activities of functional dissimilarity of soil organism communities and varied according to physical features of litter as well as their chemical quality. This research holds implication for the importance of preserving all soil fauna diversity in the sudanoguinea savannahs of Ngaoundere Cameroon for assuring efficiently nutrient cycling and contributing to soil fertility management.

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